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Hormonal Interrelationships in Postpartum Suckled Dairy Cows

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Madej A., E.O. Oyedipe, L.-E. Edqvist and H. Kindahl: Hormonal interrelationships in postpartum suckled dairy cows. Acta vet. scand. 1992, 261-271. - Three cross-bred cows calved in March and April and were followed until day 62 after parturition. Each animal was suckled by 2 calves ad libitum. All calves were removed from the cows on day 55 after parturition. Blood was collected 3 times per day from the jugular vein by venipuncture. On 4 occasions after parturition - i.e. days 7-8, 21-22, 35-36 and 49-50, the cows were bled through a jugular venous catheter every 30 min during the 24 h. The plasma samples were analyzed for the content of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (main $PGF_{2\alpha}$ metabolite), LH, prolactin, cortisol and progesterone by radioimmunoassay methods. The concentration of $PGF_{2\alpha}$ increased from 280 to 730 pmol/l within the last 4 days before parturition. The highest geometric mean was 3106 pmol/l on the day of parturition. Thereafter a steady decrease of $PGF_{2\alpha}$ metabolite concentration was seen until day 21 when it reached plateau at 148 pmo/l. In all cows plasma LH concentrations increased significantly ($P<0.05$) from about 1.6 µg/l on days 7-8 to 2.4 µg/l on days 21-22 post partum. The frequency of LH pulses showed no tendency to increase as the postpartum period progressed and averaged 6.5 pulses/24 h. Mean plasma LH concentrations increased from 2.1 µg/l 2 days before weaning to 3.2 µg/l 2 days after weaning ($P<0.05$). LH peaks occurred less frequently in association with prolactin and cortisol peaks than in their absence. A partial positive correlation between $PGF_{2\alpha}$ metabolite and cortisol ($r = 0.30$) was found on days 7-8 post partum. Correlation between prolactin and cortisol on days 7-8 and 21-22 post partum was also positive ($r = 0.20$ and $r = 0.27$, respectively). There was a negative correlation between LH and cortisol on days 7-8 ($r = -0.27$) and days 49-50 ($r = -0.21$) post partum. The first and short progesterone increase observed after weaning was terminated in conjuction with $PGF_{2\alpha}$ metabolite peaks.

PGF_{2a} metabolite; LH; prolactin; cortisol; progesterone.

Introduction

In dairy cows a large amount of the $PGF_{2\alpha}$ metabolite, 15-keto-13,14-dihydro-$PGF_{2\alpha}$, is found in the peripheral circulation from parturition until 2-3 weeks post partum (*Edqvist et al.* 1976, 1978, *Lindell et al.* 1982, *Guilbault et al.* 1984, *Madej et al.* 1984). Longer duration of the endogenous post partum prostaglandin release was related to faster involution of the uterus (*Lindell et al.* 1982, *Madej et al.* 1984) as well as to earlier resumption of ovarian activity in dairy cows (*Madej et al.* 1984). Similarly, infusion of exogenous $PGF_{2\alpha}$ during the early post partum period stimulated development of large follicles on day 15 and 35 post partum in suckled beef cows (*Villeneuve et al.* 1988).

An increase in the frequency of LH secretion, to about 0.5-1 pulse/h, is supposed to be a key endocrine event leading to resumption of cyclic ovarian activity in postpartum cows (*Carruthers & Hafs* 1980, *Peters et al.* 1981, *Schallenberger et al.* 1982, *Schallenberger* 1985). Suckling prolongs the duration of the postpartum anoestrous interval in both beef (*Carruthers & Hafs* 1980, *Gimenez et al.* 1980, *LaVoie et al.* 1981, *Humphrey et al.* 1983, *Short et al.* 1990, *Williams* 1990) and dairy cows (*Peters et al.* 1981) by suppressing episodic LH secretion. This inhibitory effect is possibly mediated through a neural pathway leading to a reduced frequency of pulsatile hypothalamic GnRH release (*Walters et al.* 1982a,b,c). The involvement of endogenous opioid peptides in the inhibition of the pulsatile LH secretion has also been forwarded (*Whisnant et al.* 1986b, *Cross et al.* 1987). No clear relationship between prolactin concentrations in blood plasma and suckling/-non-suckling endocrine effects in cows has been found (*Lamming et al.* 1981, *Wheeler et al.* 1982). It is believed that the suckling stimulus itself suppress gonadotropin secretion rather than high prolactin concentrations associated with suckling (*Williams* 1990). With respect to adrenal activity it was suggested that suckled cows had higher cortisol concentrations than milked cows (*Wagner & Li* 1982) and that cortisol, at concentrations found in suckled cows, can inhibit GnRH-induced LH release in vitro (*Padmanabhan et al.* 1983).

The following experiment was carried out to study the relationship between 15-keto-13,14-dihydro-$PGF_{2\alpha}$, LH, prolactin, cortisol and progesterone concentrations in dairy cows suckled ad libitum by 2 calves.

Materials and Methods

Animals

Three cows, crosses between the Swedish Friesian Breed and the Swedish Red and White Breed, were studied. The cows delivered single calves between March 1 and April 6, were housed in individual box stalls, and were fed according to Swedish standards (*Eriksson et al.* 1972). A foster calf less than 1 week old was introduced to each cow immediately after parturition. Both natural and foster calves suckled their dams ad libitum beginning immediately after parturition. Cows were observed for signs of oestrus at 8.00, 12.00 and 15.00 each day and were examined by rectal palpation at 7-9-day intervals to monitor follicular growth and corpus luteum development. All calves were removed from the cows on day 55 after parturition.

Blood collection

Blood was collected 3 times per day from the jugular vein by venipuncture from parturition until day 62 thereafter. On 4 occasions after parturition, days 7-8 (period 1), 21-22 (period 2), 35-36 (period 3), and 49-50 (period 4) the cows were bled through a jugular venous catheter every 30 min for 24 h. All blood samples were collected in heparinized tubes. The plasma samples were stored at -20°C until assayed.

Hormone analysis

Concentrations of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ and progesterone were determined by radioimmunoassay (*Kindahl et al.* 1976). The intra- and inter-assay coefficients of variation were 11.7% and 14.0%, respectively, for 15-keto-13,14-dihydro-$PGF_{2\alpha}$ and 8.4% and 15.0%, respectively, for progesterone.

Plasma LH concentrations were measured by homologous radioimmunoassay (*Stupnicki & Madej* 1976) with the following modifications. The incubation was carried out overnight at room temperature, including a 1h reaction between samples and antibodies prior to adding the radiolabelled LH. The bound and free fractions were separated by a second antibody against rabbit gammaglobulin coupled to a solid phase (DASP, Organon, The Netherlands). The intra- and inter-assay coefficient of variation were less than 10% and 12%, respectively.

Prolactin concentrations were determined by a double-antibody radioimmunoassay (*Schams* 1974) modified and validated by *Madej et al.* (1985). The intra-assay coefficient of variation was 10%, and the inter-assay coefficient of variation varied between 14.6% and 6.7%.

Cortisol was measured by direct radioimmunoassay using an antiserum against cortisol-21-succinyl-BSA (*Kula & Stupnicki* 1983) with modifications described by *Nyberg et al.* (1988). The intra- and inter-assay coefficients of variation were 7.0% and 9.4%, respectively.

Statistical analysis

The statistical analysis of the data was carried out with the Statistical Analysis System (*SAS Institute Inc.* 1982) using GLM and UNIVARIATE procedures and Scheffe's test. The detection of LH, prolactin, cortisol and 15-keto-13,14-dihydro-$PGF_{2\alpha}$ peaks was based on the criterion of skewness (*Christian et al.* 1978, *Brinkley* 1981). Extreme values were removed from the data set in question until the highest nonsignificant (the Shapiro-Wilks parameter) as well as until the lowest skewness was reached. The removed values were visually considered either as a peak or as the limbs of that peak. For LH, 2 types of peaks were considered. The small pulses that lasted 30 min (1 extreme value) and the large

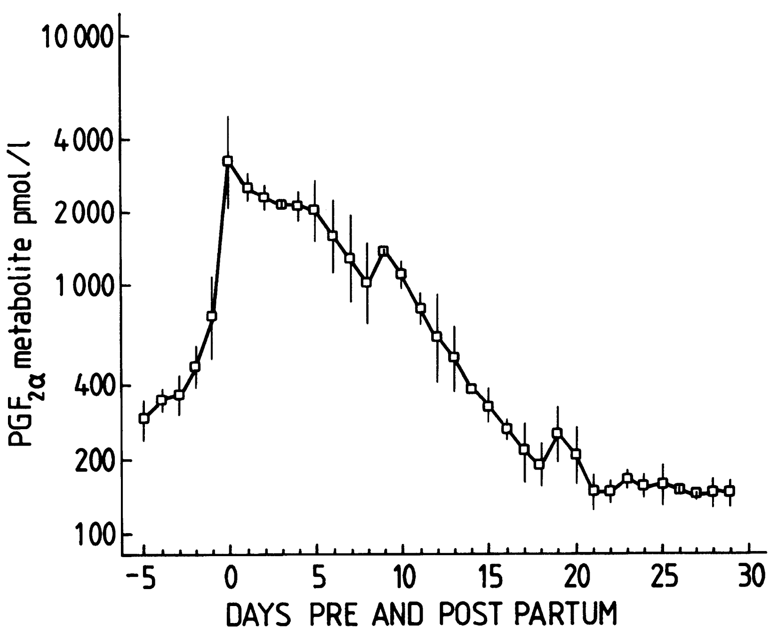

Figure 1. Geometric mean (±SEM) concentrations of $PGF_{2\alpha}$ metabolite in plasma of suckled dairy cows before and after parturition.

pulses that lasted longer than 30 min (2 or more extreme values).

Results

The cows had normal parturitions at expected times and were clinically healthy throughout the experiment. The 15-keto-13,14-dihydro-$PGF_{2\alpha}$ ($PGF_{2\alpha}$ metabolite) data from the cows were combined and log concentrations of metabolite were calculated from 5 days before parturition and through day 29 post partum (Fig. 1). The concentration of $PGF_{2\alpha}$ increased from 280 to 730 pmol/l within 4 days before parturition. The highest geometric mean was 3106 pmol/l on the day of parturition. Thereafter a steady decrease of $PGF_{2\alpha}$ metabolite concentration was seen until day 21 when it reached a plateau at 148 pmo/l.

Comparison of LH, prolactin, cortisol and $PGF_{2\alpha}$ metabolite concentrations between postpartum periods of intensive bleedings is presented in Table 1. Because of significant interaction between cows and postpartum periods all possible differencies were tested within individual cows. In all cows plasma LH increased significantly from about 1.6 µg/l on days 7-8 to 2.3-2.6 µg/l on days 21-22 and 35-36 post partum. On days 49-50 post partum 2 of the cows had mean LH values similar to those recorded on days 7-8, while 1 cow (no. 3) showed a further increase. The foster calf of cow no. 3 was unable to suckle at that time and died 9 days later. Temporal changes in the mean postpartum prolactin concentration were small. Two cows showed a significant increase of plasma cortisol between days 7-8 and days 49-50 post partum. A partial positive correlation ($r = 0.30$, $p < 0.01$) between $PGF_{2\alpha}$ metabolite and cortisol was recorded on days 7-8 post partum. The correlation between prolactin and corti-

Table 1. Mean (±SEM) concentrations of LH, prolactin, cortisol and 15-keto-13,14-dihydro-$PGF_{2\alpha}$ during 24h (n=48) sampling periods in individual suckled cows.

Cow no.	Days post-partum	LH µg/l	Prolactin µg/l	Cortisol nmol/l	$PGF_{2\alpha}$ pmol/l
1	7-8	1.57±0.06[b]	41.0±3.7[a]	3.1±0.3[c]	478±12[a]
	21-22	2.36±0.08[a]	58.2±5.7[a]	5.8±0.3[b]	140±3[bc]
	35-36	2.55±0.06[a]	61.7±8.4[a]	2.7±0.4[c]	119±2[c]
	49-50	1.90±0.13[b]	44.7±7.2[a]	11.5±0.8[a]	115±9[b]
2	7-8	1.56±0.05[c]	43.1±3.4[b]	4.1±1.0[c]	1362±31[a]
	21-22	2.37±0.06[b]	74.0±6.1[a]	4.0±0.7[c]	N.D.
	35-36	2.61±0.08[a]	37.3±4.3[b]	12.6±0.9[a]	N.D.
	49-50	1.60±0.10[c]	52.1±3.8[b]	7.4±0.4[b]	139±9[b]
3	7-8	1.78±0.08[c]	73.9±3.1[a]	6.6±1.0[ab]	2255±43[a]
	21-22	2.36±0.07[b]	39.3±4.9[b]	4.6±0.5[b]	112±2[b]
	35-36	2.29±0.08[b]	42.6±4.0[b]	4.8±0.8[b]	N.D.
	49-50*	2.91±0.12[a]	48.3±5.1[b]	9.1±1.1[a]	96±10[b]

[abc]In each cow means within the same columns with different superscripts differ (P<0.05).

*Foster calf was unable to suckle and died 9 days later.

N.D. = not determined

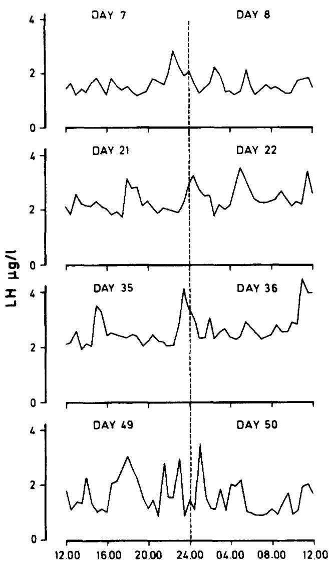

Figure 2. The 24 h profile of LH in cow no. 2 during different postpartum periods.

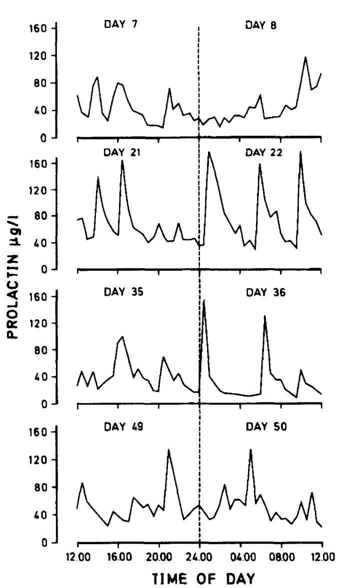

Figure 3. The 24 h profile of prolactin in cow no. 2 during different postpartum periods.

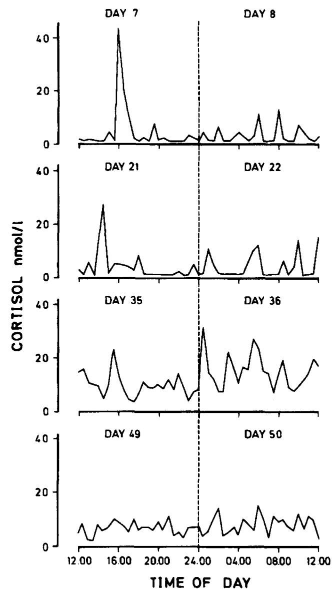

Figure 4. The 24 h profile of cortisol in cow no. 2 during different postpartum periods.

sol on days 7-8 and days 21-22 was also positive ($r = 0.20$, $p < 0.05$ and $r = 0.27$, $p < 0.01$, respectively). A negative correlation between LH and cortisol was found on days 7-8 and 49-50 ($r = -0.27$, $p < 0.05$ and $r = -0.20$, $p < 0.05$, respectively). It should be noted that these correlations were based on data originating from 30 min sampling intervals.

Temporal changes in the concentration of LH, prolactin and cortisol in cow no. 2 after parturition are shown in Figs. 2-4. The total number of LH peaks per 24 h varied between 5 and 6 during the 4 periods of intensive blood sampling from parturition to weaning (Fig. 2), and the number of so-called large LH pulses (see Material and Methods) was 3, 4, 3 and 1/24 h, respectively. Prolactin and cortisol patterns were characterized by sporadic peaks and lack of rhythmicity (Figs. 3 and 4). We found no $PGF_{2\alpha}$ metabolite peaks (Table 1) except for cow no. 3, which had a peak of 269 pmol/l, lasting 2.5 h, 49-50 days post partum.

Overall, it was found that 23 of the 32 large LH pulses occurred between 18.00 and 06.00. Of the 32 large LH pulses, 5 occurred concomitantly with peaks in prolactin as well as cortisol. Of the 47 small LH pulses, 17 and 9 occurred together with prolactin and cortisol peaks, respectively.

Fig. 5 depicts plasma LH concentrations in cow no. 2 before and after parturition based on blood samples collected 3 times per day. A gradual increase of LH concentration and an increased LH pulse frequency occurred between days 30 and 43 post partum. Overall, mean plasma LH concentrations increased from 2.1 µg/l 2 days before weaning to 3.2 µg/l 2 days after weaning ($P<0.05$). When calculating these mean values, LH concentrations higher than 10 µg/l have been omitted, since they are considered as part of the preovulatory peak.

None of the cows showed clinical signs of ovarian activity or had elevated progesterone concentrations before weaning. In cow no. 1 a short luteal phase started 5 days after weaning and was terminated 5-6 days later at which time high concentration of $PGF_{2\alpha}$ metabolite (670 pmol/l) was present (Fig. 6). Four days after weaning cow no. 2 exhibited an LH peak of a magnitude resembling that of a preovulatory LH surge (Fig. 5) and afterwards a shortlasting progesterone increase was seen (Fig. 6). The short luteal phase lasted about 5 days and progesterone returned to baseline concomitantly with an

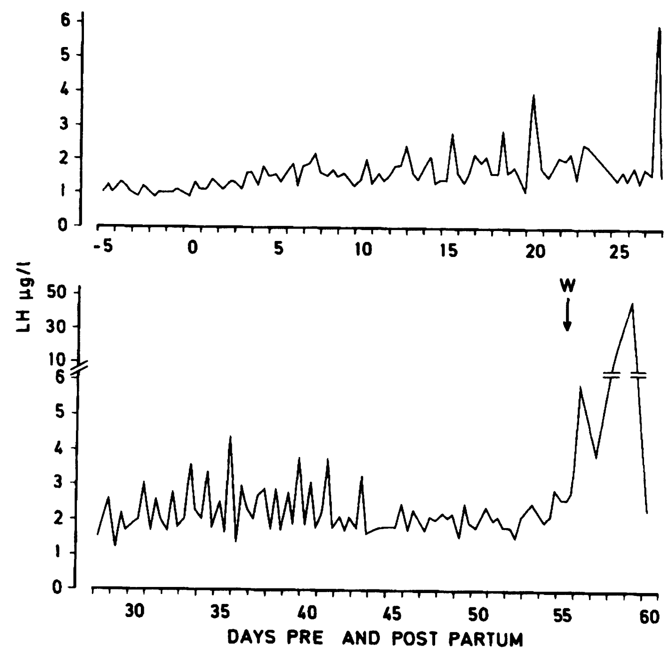

Figure 5. Plasma LH concentrations in cow no. 2 from day 5 before parturition to day 60 post partum. Note: W = day of weaning.

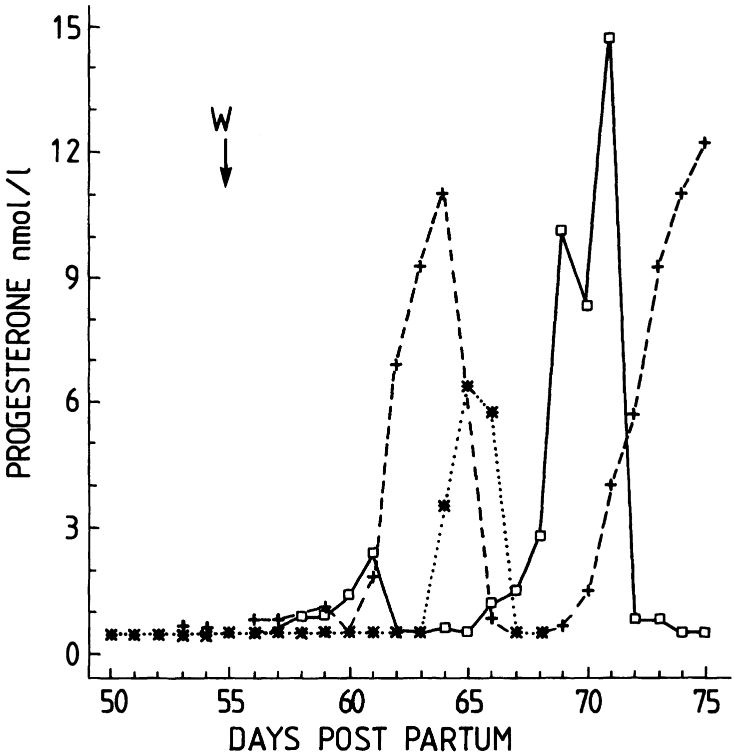

Figure 6. Plasma progesterone concentrations in cow no. 1 (+ --- +), cow no.2 (* .... *) and cow no. 3 (□——□) before and after weaning (W).

increased concentration of $PGF_{2\alpha}$ metabolite (510 pmol/l). In cow no. 3 the transient increase of progesterone concentration to 2.4 nmol/l (Fig. 6) was seen together with a marked elevation of $PGF_{2\alpha}$ metabolite (453 pmol/l). Then, 1 week later a new increase of progesterone which lasted 6 days was recorded.

Discussion

Postpartum prostaglandin $F_{2\alpha}$ release as reflected by the concentration of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ lasted 21 days in the suckled dairy cows studied here. This is a few days longer than we have recorded in milked cows of the same breed (*Madej et al.* 1986). Furthermore, *Troxel el al.* (1984) reported that concentrations of $PGF_{2\alpha}$ metabolite peaked on day 4 postpartum and then declined to reach baseline on day 22 postpartum in the suckled beef cow. Collectively, these results suggest that the postpartum elevation of $PGF_{2\alpha}$ in suckled cows may last a few days longer than in milked cows.

The changes in plasma LH concentrations found here during the postpartum period are in agreement with the findings of *Carruthers & Hafs* (1980), *Rawlings et al.* (1980) and *Garcia-Winder et al.* (1984). Thus the mean frequency of LH peaks (6.5/24 h) is very similar to that reported by *Carruthers et al.* (1980) and *Garcia-Winder et al.* (1984). This low frequency of LH peaks in suckled cows might be associated with ovarian inactivity during suckling. *Fisher et al.* (1986) found no differences in either LH or oestradiol-17ß secretion between milked and suckled cows. Instead, these authors reported a higher LH pulse frequency in cows with short duration of post partum anestrus than in cows with long duration of post partum anestrus.

When the calves were removed from their dams we observed an increase in LH concentrations similar to that previously reported by *Whisnant et al.* (1985) and *Edwards* (1985).

The negative correlation between LH and cortisol accords with findings of *Gregg et al.* (1986), who reported cortisol concentrations to be lower in cows responding to naloxone than in cows not responding to this treatment. In contrast, *Whisnant et al.* (1985) concluded that cortisol may not be a physiological inhibitor of LH secretion in the suckled postpartum beef cow. *Whisnant et al.* (1986a) found that larger doses of naloxone were required to increase serum LH on day 14 postpartum compared with days 28 and 42 post partum. Thus, they suggested that opioid inhibition of LH secretion may change during the postpartum period in suckled beef cows. This is supported by the results obtained here in which a negative correlation between LH and cortisol was documented on days 7-8 and days 49-50 but not on days 21-22 and 35-36 post partum.

The stressful conditions of experimentation might also be involved in the mechanism responsible for relations between LH and cortisol (*Gregg et al.* 1986). However, *Alam & Dobson* (1986) found no evidence for suppression of tonic LH release in cycling cows after the commonly practised veterinary manipulations that caused increased plasma cortisol concentration.

The positive correlations between 15-keto-13,14-dihydro-$PGF_{2\alpha}$ and cortisol and between prolactin and cortisol resemble the correlations found in milked cows (*Madej et al.* 1986).

There was neither rhythmicity nor a pulsatile pattern of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ in our suckled cows. This is in agreement with *Basu & Kindahl* (1987) who reported neither rhythmicity nor $PGF_{2\alpha}$ metabolite peaks during early pregnancy in blood plasma continuously collected from heifers.

The non-coincidental occurrence of LH and prolactin peaks in the present study accords with previous studies. For instance, *Gimenez et al.* (1980) found that only 23% of the LH peaks occurred during prolactin peaks. In the present study we also found a similar relation between LH and cortisol peaks.

Upon weaning all cows experienced elevated progesterone concentrations of short duration. This shortlasting progesterone increase is likely the result of an ovulation since *Eger et al.* (1988) concluded that if short luteal cycles occur in healthy cows they can be attributed to a functional corpus luteum and not to ovary malfunction. Evidently, the short progesterone increase observed was terminated in conjuction with $PGF_{2\alpha}$ metabolite peaks. This is similar to the situation in milked dairy cows resuming sexual functions post partum. Also in this situation short lasting progesterone elevations terminated by the release of $PGF_{2\alpha}$ is seen (*Kindahl et al.* 1984, *Madej et al.* 1986). This is also consistent with a recent report demonstrating that short luteal cycles appear to be associated with a premature release of $PGF_{2\alpha}$ from the endometrium of the uterus (*Zollers et al.* 1991).

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Sammanfattning

Hormonförändringar hos digivande kor efter förlossningen.

På 3 kor som var diade av 2 kalvar studerades de perifera nivåerna av 15-keto-13,14-dihydro-$PGF_{2\alpha}$, LH, prolaktin, kortisol och progesteron från förlossningen till 7 dagar efter avvänjningen dvs 62 dagar post partum. Blodprover samlades 3 gånger per dag. Dessutom togs blodprov via permanent jugularven kateter var 30:e minut under 24 timmar dag 7-8, 21-22, 35-36 och 49-50. Cervix, uterus och äggstockar undersöktes genom rektalpalpation var 7-9 dag. Ingen ko visade brunst under diperioden. Frisättningen av $PGF_{2\alpha}$ post partum varade 21 dagar. LH-koncentration steg från 1,6 µg/l på dag 7-8 till 2,4 µg/l på dag 21-22 post partum. Det påvisades en signifikant positiv korrelation mellan $PGF_{2\alpha}$ och kortisol (dag 7-8) liksom mellan prolaktin och kortisol (dag 7-8 samt dag 21-22). Vidare noterades en signifikant negativ korrelation mellan LH och kortisol (dag 7-8 samt dag 49-50). Efter avvänjningen steg progesteronproduktionen och en kort lutealfas utvecklades. Denna avslutades genom frisättning av $PGF_{2\alpha}$.

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